

Atty. Docket No.: P65141US0  
Serial No.: 09/508,095

- application of a cation-exchange HPLC step;
- collecting fractions;
- adjusting the fractions to a salt content of < 25 mM by dialysis or reverse phase HPLC for performing activity tests;
- culturing *Bifidobacterium bifidum* and *E. coli* in the presence of the fractions and selecting fractions which meet the requirement:

$$\frac{BW}{B0} - \frac{EW}{E0} \geq 0.15 \text{ (bifidogenic)}$$

wherein

- C2
- BW represents the germ count obtained upon 16 hours of incubation of *Bifidobacterium bifidum* in 50% Elliker broth in the presence of the peptides in a concentration of 200 µg/ml;
  - B0 represents the germ count obtained in the control incubation without active substances;
  - EW represents the germ count obtained upon 16 hours of incubation of *E. coli* in 3 g/l tryptic soy broth in the presence of the peptides in a concentration of 200 µg/ml;
  - E0 represents the germ count obtained in the control incubation without active substances; and
  - isolation of the peptides contained in this fraction;

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amidated, acetylated, sulfated, phosphorylated, glycosylated, or oxidized derivatives of the peptides, the derivatives having bifidogenic properties, fragments of the peptides, the fragments having bifidogenic properties, and combination peptides obtainable by chemically bonding the peptides, the amidated, acetylated, sulfated, phosphorylated, glycosylated, or oxidized derivatives having bifidogenic properties of the peptides, or the fragments having bifidogenic properties.

7. A peptide having amino acid sequence

*C2*

$R_1$ -EQLRLKK- $R_2$ ,  $R_1$ -YLEQLRLKKY- $R_2$ ,  $R_1$ -NRQRNILR- $R_2$ ,  
 $R_1$ -YMNGMNRQRNILR- $R_2$ ,  $R_1$ -FQWQNMNRK- $R_2$ ,  $R_1$ -HTGLRRTA- $R_2$ ,  
 $R_1$ -FTAIQNLRK- $R_2$ ,  $R_1$ -EVAARARVW- $R_2$ ,  $R_1$ -WQNMNRKV- $R_2$ ,  
 $R_1$ -LARPLKRLK- $R_2$ ,  $R_1$ -YKQKVEKV- $R_2$ ,  $R_1$ -LVRYTKKV- $R_2$ ,  
 $R_1$ -KYLVEIARR- $R_2$ ,  $R_1$ -ARRARVVWCAVG- $R_2$ ,  $R_1$ -ARRARVVWCAVGE- $R_2$ ,  
 $R_2$ -CIAL- $R_4$   $R_3$ -CIAL- $R_4$   
 $R_1$ -YQRRPAIAINNPYVPRTYANPAVVRPHAQIPQRYLPNSHPPTVVRRPNLHPSF- $R_2$ ,  
 $R_1$ -GRRRSVQWCIVSQPEATKCFQWQNMNRVVRGPPVSCIQRDSPICIQ- $R_2$ ,  
 $R_1$ -GRRRSVQWCAVSQPEATKCFQWQNMNRKVVRGPPVSCIQRDSPICIQ- $R_2$ ,  
 $R_1$ -GRRRSVQWCAVSQPEATKCFQWQNMNRKVVRGPPVSCIQRDSPICIQ- $R$ ,  
 $R_1$ -VYQHOKAMPKFWIQPKTKVIPYVRYL- $R_2$ ,  $R_1$ -ARRARVVWAAVG- $R_2$ ,  
 $R_1$ -CAVGGGCIAL- $R_2$ , or  
 $R_1$ -RHTRKYWCROGARGGCITL- $R_2$ ,

wherein

$R_1$ ,  $R_2$  independently represents  $NH_2$ , an amino acid, or a peptide containing up to 100 amino acids, and

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$R_2, R_4$  independently represents  $\text{COOH}$ ,  $\text{CONH}_2$ , an amino acid, or a peptide containing up to 100 amino acids;

the peptide N-modified by amidation, acetylation, sulfation, phosphorylation, glycosylation, or oxidation having bifidogenic properties, or a fragment thereof having bifidogenic properties.

8. The peptide of claim 6 having the amino acid sequence SEQ ID NO: 10, SEQ ID NO: 22, or SEQ ID NO: 23.

C2 9. A method of obtaining peptides comprising the steps of:

- adding proteases to cow's milk, or human milk, followed by incubation for two hours;
- centrifugation to remove milk fat;
- acidification to a pH of 2.0 with strong acids;
- removing the precipitated proteins;
- application of at least one reverse phase HPLC step;
- application of a cation-exchange HPLC step;
- collecting fractions;
- adjusting the fractions to a salt content of < 25 mM by dialysis or reverse phase HPLC for performing activity tests;
- culturing *Bifidobacterium bifidum* and *E. coli* in the presence of the fractions and selecting fractions which meet the requirement;

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  - EW represents the germ count obtained upon 16 hours of incubation of *E. coli* in 3 g/l tryptic soy broth in the presence of the peptides in a concentration of 200 µg/ml;
  - E0 represents the germ count obtained in the control incubation without active substances; and
  - isolation of the peptides contained in this fraction.
10. The method of claim 9, further comprising the step of obtaining amidated, acetylated, sulfated, phosphorylated, glycosylated, or oxidized derivatives or fragments of the isolated peptide having bifidogenic properties.